

Amendments to the Claims

1. (Currently amended) A method of detecting target nucleic acid sequences, the method comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes, wherein each structured probe is bifunctional, wherein one function is as a probe to a target nucleic acid sequence and the other function is a detection function, wherein the detection function is not possible unless the probe hybridizes to the target nucleic acid sequence,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes,

wherein, prior to detection, the structured probes are treated to alter ~~or eliminate~~ unhybridized probes,

wherein only probes with intact duplex regions are altered ~~or eliminated~~ by treatment of the structured probes, wherein altered ~~and eliminated~~ probes are not detected.

2. (Currently amended) The method of claim 1 wherein the second complementary portion of at least one probe is at the second end of the probe, wherein the first complementary portion is not at the first end of the probe,

wherein the treatment is extending the second end of the probe using the portion of the probe closer to the first end of the probe than the first complementary portion as template, wherein only probes with intact duplex regions~~stems~~ are extended.

3. (Original) The method of claim 2 wherein the second end sequence of the extended probe is not complementary to the primer complement portion of an amplification target circle and cannot prime replication of the amplification target circle.

4. (Original) The method of claim 2 wherein during extension the second end of the probe is capped by addition of a chain-terminating nucleotide such that the probe cannot prime nucleic acid synthesis.

5. (Original) The method of claim 2 wherein the target nucleic acid sequences are associated with a solid support, wherein hybridization between target nucleic acid sequences in the target samples and the structured probes associates the probe with the solid support.

6. (Original) The method of claim 2 wherein the probes are associated with a solid support.

7. (Original) The method of claim 6 wherein the first ends of the probes are coupled to the solid support.

8. (Original) The method of claim 2 wherein a portion of the detection portion extends beyond the second complementary portion toward the first end and is in the loop.

9. (Original) The method of claim 2 wherein the detection portion and the second complementary portion are the same portion of the probe.

10. (Original) The method of claim 2 wherein extension of the second end of the probe makes the detection portion non-functional, reduces or eliminates the detectability of the detection portion, or a combination.

11. (Original) The method of claim 1 wherein the duplex region of at least one probe comprises a nucleic acid cleavage site, wherein the treatment is cleavage of the nucleic acid cleavage site, wherein only probes with intact duplex regions are cleaved.

12. (Original) The method of claim 11 wherein the detection portion does not overlap the nucleic acid cleavage site such that the nucleic acid cleavage site is closer to the first end of the probe than the detection portion.

13. (Currently amended) The method of claim 11 wherein the detection portion does not overlap the second complementary portion such that the second complementary portion is closer

to the first end of the probe than the detection portion, wherein the duplex region~~stem~~ comprises a nucleic acid cleavage site.

14. (Original) The method of claim 11 wherein the nucleic acid cleavage site is a restriction site, wherein the site is cleaved with a restriction enzyme.

15. (Original) The method of claim 11 wherein the probes are associated with a solid support.

16. (Original) The method of claim 15 wherein the first ends of the probes are coupled to the solid support.

17. (Original) The method of claim 11 wherein the detection portion partially overlaps the second complementary portion.

18. (Original) The method of claim 17 wherein the second complementary portion is not at the second end of the probe.

19. (Original) The method of claim 11 wherein cleavage of the nucleic acid cleavage site eliminates the detection portion from the probe, makes the detection portion non-functional, reduces or eliminates the detectability of the detection portion, or a combination.

Claims 20-26. (Cancelled).

27. (Original) The method of claim 1 wherein structured probes with intact duplex regions are not detected.

28. (Original) The method of claim 27 wherein the detection portion and the second complementary portion are the same portion of the probe.

29. (Currently amended) The method of claim 27 wherein a portion of the detection portion extends beyond the second complementary portion toward the first end and is in the loop, wherein hybridization of an amplification target circle to the portion of the detection portion that is in the loop will not disrupt the duplex region~~stem~~.

30. (Original) The method of claim 27 wherein the target nucleic acid sequences are associated with a solid support, wherein hybridization between target nucleic acid sequences in the target samples and the structured probes associates the probe with the solid support.

31. (Original) The method of claim 1 wherein the duplex region and loop is a hairpin stem and loop structure.

32. (Original) The method of claim 31 wherein the first end of the structured probe is a 5' end and the second end of the structured probe is a 3' end.

33. (Original) The method of claim 32 wherein the second complementary portion, the first complementary portion, the target probe portion, and the detection portion are each in a 5' to 3' orientation relative to the first end.

34. (Original) The method of claim 1 wherein the duplex region and loop is a curl stem and loop structure.

35. (Original) The method of claim 34 wherein the first end of the structured probe is a 3' end and the second end of the structured probe is a 3' end.

36. (Original) The method of claim 35 wherein there is a 5' to 5' junction between (a) the second complementary portion and detection portion and (b) the target probe portion.

37. (Original) The method of claim 36 wherein the second complementary portion and detection portion are each in a 3' to 5' orientation relative to the second end, and wherein the target probe portion and first complementary portion are each in a 3' to 5' orientation relative to the first end.

38. (Original) The method of claim 35 wherein there is a 5' to 5' junction between the target probe portion and the first complementary portion.

39. (Original) The method of claim 38 wherein the second complementary portion, detection portion, and target probe portion are each in a 3' to 5' orientation relative to the second end, and wherein the first complementary portion is in a 3' to 5' orientation relative to the first end.

40. (Original) The method of claim 1 wherein the detection portion comprises a rolling circle replication primer.

41. (Original) The method of claim 40 wherein detection of the structured probes is accomplished by,

bringing into contact one or more amplification target circles and the structured probes, and incubating under conditions that promote hybridization of the amplification target circles to the structured probes,

incubating the amplification target circles and the structured probes under conditions that promote replication of the amplification target circles,

wherein replication of the amplification target circles results in the formation of tandem sequence DNA.

42. (Currently amended) The method of claim 41 wherein, prior to detection, the structured probes are treated to alter ~~or eliminate~~ unhybridized probes,

wherein only probes with intact duplex regions are altered ~~or eliminated~~ by treatment of the structured probes, wherein altered ~~and eliminated~~ probes do not prime replication of the amplification target circles.

43. (Original) The method of claim 42 wherein structured probes with intact duplex regions do not prime replication of the amplification target circles.

44. (Original) The method of claim 1 wherein at least one of the target nucleic acid sequences or at least one of the structured probes is associated with a solid support.

45. (Original) The method of claim 44 wherein the first ends of the probes are coupled to the solid support.

46. (Original) The method of claim 44 wherein each of the target nucleic acid sequences or structured probes is located in a different predefined region of the solid support.

47. (Original) The method of claim 46 wherein the distance between the different predefined regions of the solid support is fixed.

48. (Original) The method of claim 47 wherein the solid support comprises thin film, membrane, bottles, dishes, fibers, woven fibers, shaped polymers, particles, beads, microparticles, or a combination.

49. (Original) The method of claim 46 wherein the distance between at least two of the different predefined regions of the solid support is variable.

50. (Original) The method of claim 49 wherein the solid support comprises at least one thin film, membrane, bottle, dish, fiber, woven fiber, shaped polymer, particle, bead, or microparticle.

51. (Original) The method of claim 50 wherein the solid support comprises at least two thin films, membranes, bottles, dishes, fibers, woven fibers, shaped polymers, particles, beads, microparticles, or a combination.

52. (Original) The method of claim 46 wherein each of the target nucleic acid sequences is located in a different predefined region of the solid support, wherein the location on the solid support where the structured probes are detected indicates the presence in the target sample of the target nucleic acid sequence corresponding to that region of the solid support.

53. (Original) The method of claim 46 wherein each of the structured probes is located in a different predefined region of the solid support, wherein the location on the solid support where the structured probes are detected indicates the presence in the target sample of the target nucleic acid sequence corresponding to the structured probe at that region of the solid support.

54. (Original) The method of claim 44 wherein the solid support comprises a plurality of target nucleic acid sequences located in a plurality of different predefined regions of the solid support, wherein the target nucleic acid sequences collectively correspond to a plurality of structured probes.

55. (Original) The method of claim 44 wherein the solid support comprises a plurality of structured probes located in a plurality of different predefined regions of the solid support, wherein the structured probes collectively correspond to a plurality of target nucleic acid sequences.

56. (Original) The method of claim 44 wherein the solid support comprises thin film, membrane, bottles, dishes, fibers, woven fibers, shaped polymers, particles, beads, microparticles, or a combination.

57. (Original) The method of claim 44 wherein the solid support comprises acrylamide, agarose, cellulose, nitrocellulose, glass, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfumerate, collagen, glycosaminoglycans, or polyamino acids.

58. (Original) The method of claim 44 wherein the solid support is porous.

59. (Original) The method of claim 1 wherein the target probe portion will not hybridize to a mismatched nucleic acid sequence, wherein the mismatched nucleic acid sequence will not disrupt the duplex region.

60. (New) The method of claim 1 wherein the treatment is extending the second end of the probe using probe sequence as template.

61. (New) The method of claim 60 wherein the duplex region is a curl stem and loop structure.

62. (New) A method of detecting target nucleic acid sequences, the method comprising
(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes, wherein each structured probe is bifunctional, wherein one function is as a probe to a target nucleic acid sequence and the other function is a detection function, wherein the detection function is not possible unless the probe hybridizes to the target nucleic acid sequence,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes,

wherein, prior to detection, the structured probes are treated to alter unhybridized probes, wherein the treatment is extending the second end of the probe using probe sequence as template, wherein only probes with intact duplex regions are altered by treatment of the structured probes, wherein altered probes are not detected.

63. (New) A method of detecting target nucleic acid sequences, the method comprising

(a) bringing into contact one or more target samples and one or more structured probes, and incubating under conditions that promote hybridization between target nucleic acid sequences in the target samples and the structured probes, wherein each structured probe is bifunctional, wherein one function is as a probe to a target nucleic acid sequence and the other function is a detection function, wherein the detection function is not possible unless the probe hybridizes to the target nucleic acid sequence,

wherein each structured probe has a first end and a second end, wherein each structured probe comprises at least two complementary portions, a target probe portion, and a detection portion, wherein the two or more complementary portions comprise a first complementary portion and a second complementary portion,

wherein two or more of the complementary portions interact with each other to form a duplex region, wherein formation of the duplex region forms a loop, wherein at least a portion of the target probe portion is in the loop, wherein hybridization of the target probe portion to the target nucleic acid sequence disrupts the duplex region,

(b) detecting the structured probes,

wherein, prior to detection, the structured probes are treated to alter unhybridized probes,

wherein only probes with the duplex region intact are altered by the treatment of the structured probes, whereby altered probes are not detected.